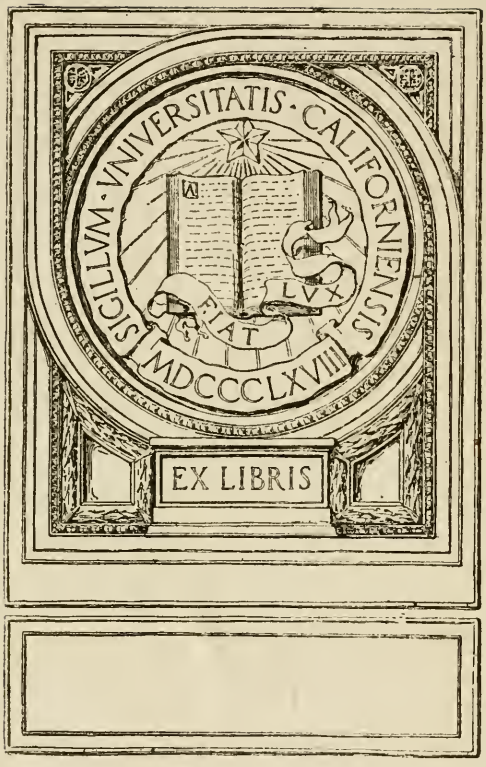
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STUDIES ON THE DECOMPOSITION OF CELLULOSE IN SOILS

BY

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Introduction

The discovery and comprehension of the biological and chemical forces relating to the decomposition of the carbohydrate materials in soils is unquestionably necessary to the solution of many problems in soil fertility and crop production. A large percentage of the carbon content of the plant residue is found as a constituent of the celluloses. These compounds, because of their refractory nature, must first be attacked by a special group of organisms. An accurate knowledge of the cultural and biochemical characteristics of the organisms involved in the transformation of cellulose into less refractory compounds is, therefore, obviously of the greatest importance.

Extensive investigations during the last few years have shown that the decomposition of cellulose is by no means limited to the bacteria of soils. The filamentous fungi possessing this power are very numerous and many species are exceedingly active agents in the destruction of cellulose. In the humid soils of the East the filamentous fungi are perhaps of greater importance than bacteria in the destruction of cellulose, while in the semi-arid soils of the West the reverse is apparently true. Several species of Actinomyces are also known to have the power of dissolving cellulose and because of their general distribution, these organisms are undoubtedly a factor in the destruction of cellulose in soils. This paper deals, for the most part, with investigations of cellulose-dissolving bacteria.

Culture Media

Methods for the preparation of cellulose agar and other suitable culture media for the study of cellulose-dissolving bacteria have been discussed, at some length, in earlier publications by Kellerman and McBeth (29), McBeth and Scales (43), Löhnis and Lochhead (40), Kellerman,

[1] Paper No. 16, Citrus Experiment Station, College of Agriculture, University of California, Riverside, California.

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A bibliography of the literature relating to cellulose destruction is included and reference is made by numbers to "literature cited" (p. 481).

McBeth, Scales, and Smith (30), and Scales (71). The cellulose agar prepared as described in the above mentioned publications has given very satisfactory results not only with cellulose-dissolving bacteria, but also with filamentous fungi. The medium also appears to be well adapted to the study of cellulose destruction by species of Actinomyces. We have frequently observed colonies of Actinomyces which dissolve the cellulose very rapidly in the cellulose agar, forming a clear enzymic zone about the colony which furnishes unmistakable evidence of the cellulose-dissolving power of the organism. Krainsky (36) in his recent studies of the Actinomyces, has reported the cellulose agar plate method as unsatisfactory for determining the cellulose-dissolving power of these organisms. However, he was able to demonstrate the cellulose-dissolving power of several species of Actinomyces by the use of paper pulp or strips of paper on silica jelly, and also by means of cellulose hydrate prepared by the zinc chloride method. The reason for the failure of Krainsky to secure satisfactory results with the cellulose agar plate method is not clear. However, since these organisms grew luxuriantly upon the cellulose agar prepared by us and dissolved the cellulose very rapidly, it would seem that the unsatisfactory results reported by Krainsky may be due to certain inattention to details in the preparation of the cellulose precipitate. In order to secure a uniformly fine amorphous precipitate, it is necessary to carry out the operations with considerable care. It is believed that much of the difficulty experienced in the preparation of precipitated cellulose is caused by precipitating in solutions that are too concentrated. If either the copper-ammonium-cellulose solution or the acid used in precipitating the cellulose is too concentrated, a product is frequently secured which is not only difficult to wash, but is very unsatisfactory as a culture medium. A very uniform and satisfactory amorphous precipitate can be secured by adhering strictly to the following method which is a slight modification of the method originally proposed.

1. Pour 1 liter of ammonium hydroxide, sp. gr. 0.90, into a glass-stoppered bottle; add 250 c.c. of distilled water and 75 gm. of pure copper carbonate; shake the solution vigorously until all the copper is dissolved. (From 10 to 15 minutes is ordinarily required.)

2. To the copper-ammonium solution add 15 gm. of high grade, sheet filter paper; shake vigorously at intervals of 10 minutes for one-half hour. Examine the solution carefully to see that the paper is completely dissolved. If any particles of paper remain in the solution, the shaking must be continued until the solution is perfectly clear.

Dilute 250 c.c. of the ammonium-copper-cellulose solution to 10 liters with tap water; add slowly with frequent shaking, a weak hydrocloric acid solution prepared by adding 500 c.c. of concentrated acid to 10 liters of

tap water. Continue the addition of the acid until the blue color disappears; add a slight excess of acid, shake thoroughly and allow to stand a few minutes. The finely precipitated cellulose will rise to the top, due to the large quantity of free hydrogen liberated in the precipitation process. Shake the solution vigorously at intervals of a few minutes to dislodge the hydrogen. As soon as the free hydrogen has escaped the cellulose will settle rapidly.

3. Wash through repeated changes of water until free from copper and chlorine. After the washing is complete, bring the cellulose in the solution up to 0.5 per cent, by allowing to settle a few days and siphoning off the clear solution or by evaporating. Add the nutrient salts desired together with 1 per cent of thoroughly washed agar; heat in autoclave or boil until the agar is dissolved; tube and sterilize in the usual way.

Action of the Cellulose-Dissolving Bacteria Studied on the Cellulose of Plant Tissues

While the preparation of cellulose agar from precipitated cellulose as described above has proven quite satisfactory for the isolation and study of organisms which dissolve typical cellulose, such as is found in filter paper or in cotton fiber, it does not make possible a study of the action of the organisms on the celluloses in plant tissues such as are ordinarily added to the soil, as stubble, roots, green manure, etc. Since the term "cellulose" connotes a group of substances rather than a single chemical compound, it seems important that methods be devised which will make possible a comparative study of the action of the cellulose-dissolving organisms isolated from the soil, upon the cellulose of different plants and also of the same plants at different stages of maturity. In the young plant cells the walls contain almost pure cellulose, but as the plant developes the cellulose originally formed is altered by the addition to it of various secondary products known as encrusting substances. The nature and properties of the resulting fiber depends, of course, upon the nature of the substances deposited.

Since many of the cellulose-dissolving organisms attack not only the celluloses, but many other plant substances such as the starches, sugars, and proteins, it is necessary in studying the action of these organisms on the cellulose of different plant tissues, other than that of cotton fiber, to separate the cellulose from the other compounds with which it is more or less closely associated in the plant. It is also important that the purified cellulose be separated into very fine particles such as will permit the preparation of a satisfactory cellulose agar. Finely divided pure cellulose suitable for the preparation of cellulose agar may be prepared from plant substances as follows:

1. Grind a quantity of the dry plant substance to a flour and sift through bolting cloth to remove all coarse material.

2. Boil 50 gm. of the sifted flour in a 2 per cent potassium hydrate solution for one-half hour; pour into a large bottle or carboy and wash through repeated changes of water until free from potassium.

3. Expose the washed material to the action of chlorine at ordinary temperatures for one-half hour. Wash as before until the chlorine is removed.

4. Subject to a second alkaline hydrolysis by boiling with 2 per cent caustic soda for one-half hour. Wash until the solution is no longer alkaline.

The cellulose is thus isolated in a very pure state, and if the grinding of the plant material has been sufficiently fine, the finely divided cellulose prepared in this way is quite as satisfactory for the preparation of cellulose agar as that prepared from filter paper by the ordinary method.

In the present work it has not been possible to make an extensive study of the decomposition of the celluloses in different plant substances. However, it has been demonstrated that the cellulose-dissolving bacteria isolated from soils by means of the cellulose agar plate method, have the power of dissolving the cellulose of alfalfa. Twenty-five species of cellulose-dissolving bacteria were plated to cellulose agar containing pure cellulose from the alfalfa plant and in every instance the cellulose was dissolved as readily as that prepared from filter paper by the ordinary method.

Discussion of General Characteristics of Cellulose-Dissolving Bacteria

The author's exhaustive studies of a large number of soils from widely separated regions have shown that there are numerous species of bacteria which have the power to destroy cellulose. All of the forms studied are rod-shaped organisms varying in length from .8 to 3.50 μ. Involution forms have been observed for only three species. Five species have been found to produce spores. Twenty-seven of the thirty-six species isolated are motile. The arrangement of the flagella on the motile forms shows that seven species belong to the genus Pseudomonas and twenty to the genus Bacillus. All species stain readily with the aniline dyes. All are facultative in nature, but invariably develop most rapidly under aerobic conditions. With some species, the development under anaerobic conditions is very slow. All species grow well from 20° to 37.5° C., and some forms have been found to develop at temperatures as high as 45° C., but much more slowly than at the lower temperatures. The optimum temperature for most species seems to lie between 28° to 33° C.

With two exceptions, the cellulose-destroying bacteria form more or less growth upon ordinary culture media such as beef gelatin, beef agar,

etc. Of the thirty-four species which grow upon gelatin, nineteen liquefy the gelatin. Many forms produce a growth upon beef agar and potato agar slopes in 24 hours. A few species grow quite luxuriantly upon potato cylinders, but in most cases no growth or only a scant growth is produced, even when the cultures are held in a moist chamber for 30 days. Twenty-nine species produce an acid reaction and three an alkaline reaction in litmus milk. Four species do not change the reaction of litmus milk. The milk is coagulated or digested by only six species.

The destruction of cellulose can be secured in nutrient solutions containing ammonium sulphate, potassium nitrate, peptone, casein, or asparagin as the source of nitrogen. Peptone appears to give the best results for the largest number of organisms, while casein is least satisfactory for many forms. No destruction of cellulose has been secured without the addition of combined nitrogen to the nutrient solution. This would seem to indicate that the cellulose-dissolving organisms do not draw freely upon the free nitrogen of the air for their nitrogen supply. This hypothesis is further strengthened by the behavior of the organisms in dextrose solutions. When dextrose is added to nutrient solutions containing combined nitrogen, many of the cellulose-dissolving organisms vigorously attack the dextrose; but when the nutrient solution is carefully freed from combined nitrogen the dextrose is attacked very slowly and little or no fixation of nitrogen is secured.

No gas is formed by any of the species in cellulose or other carbohydrate broths. The quantity of acid produced in carbohydrate broths is fairly constant for the species, but quite variable for different species. With dextrose, lactose, maltose, saccharose, and starch the quantity of acid produced in 12 days at 30° C. usually lies between 1 and 2 per cent on Fuller's Scale. The amount of acidity in the mannite and glycerine solutions is very generally less than 1 per cent, and in many cases no acidity is produced in these solutions. Two species cause no change in the reaction of any of the carbohydrate broths. *B. rossicus* gave an alkaline reaction in all the broths, while *Ps. effusa* gave an alkaline reaction in the lactose and saccharose broths. The alkaline reaction is probably due to the formation of ammonia from the peptone in the solution, the ammonia produced being more than sufficient to neutralize any acid formed. In Dunham's solution fourteen species produce ammonia, while twenty forms produce a compound which gives typical reactions for nitrites with the Griess' reagent and also with the starch-iodide and the diphenylamine solutions. There seems to be no reason for concluding that the substance is not nitrite except that nitrite formation has been thought to be restricted to a particular group of organisms which do not grow upon ordinary media. The quantity of nitrite formed by the cellulose-dissolving forms is small; in most instances not more than one

part per million of nitrogen as nitrite is produced. However, the formation of this small amount is constant and is, therefore, of considerable value as a diagnostic feature.

Since many species produce nitrites in Dunham's solution, it is obvious that erroneous conclusions might be drawn from the use of a nitrate broth containing peptone. Peptone has therefore been left out of the nitrate broth used in studying the nitrate reducing power of these organisms, and a small quantity of starch added to furnish the necessary carbon. In this broth many of the species reduce nitrates to nitrites, but only four forms reduce nitrates to ammonia.

The Occurrence and Activity of Cellulose-Dissolving Bacteria in Southern California Soils

Examinations of 69 soils of southern California for cellulose-dissolving bacteria indicate that these soils contain numerous species of bacteria which have the power of dissolving cellulose. All of the soils examined were found to contain one or more active cellulose-destroying forms and most of the species isolated were found in two or more soils from widely separated districts. One of the most active forms *(B. imminutus)* was isolated from ten of the sixty-nine soils examined. From the southern California soils studied fifteen new species of cellulose-dissolving bacteria have been isolated and described. In addition to the new species found, seven species previously isolated from other soils have been identified. The distribution of the cellulose-dissolving bacteria found in the southern California soils is shown in Table I.

It is well known that a very rapid destruction of cellulose occurs in many citrus soils of southern Colifornia. The question naturally arises whether the rapid destruction of cellulose in these soils is due to the presence of unusually active cellulose-destroying organisms or to favorable conditions which make possible a very rapid multiplication of the cellulose-dissolving organisms present. From the studies made, it is evident that the soils are abundantly supplied with active cellulose-destroying bacteria. Moreover, some of the most active forms appear to have a very wide distribution in the soils of southern California. However, with the possible exception of *B. imminutus* the cellulose-destroying bacteria found in southern California soils, when placed under standard conditions, appear to be no more active agents in the destruction of cellulose than the organisms isolated from the humid regions of the United States. In any explanation of the rapid destruction of cellulose in these soils we must take into consideration the activity of filamentous fungi and possibly the Actinomyces. The cellulose-destroying fungi are unquestionably less numerous and less active in the semi-arid soils of southern California than in the humid soils of the eastern part of the United States. The same is apparently true of the cellulose-destroying species of Actinomyces.

TABLE I

DISTRIBUTION OF CELLULOSE-DISSOLVING BACTERIA IN SOUTHERN CALIFORNIA SOILS

		Arlington	Azusa	Banning	Barstow	Berkeley	Bonita	Chula Vista	Corona	Covina	Davis	Fullerton	Glendora	Highgrove	Highland	Lordsburg	Mentone	Monrovia	Pasadena	Porterville	Redlands	Riverside	San Fernando	Sanger	Santa Paula	Tustin	Uplands	Whittier	Wineville
B. albidus																										*			
B. almus		*					*												*										
B. concitatus					*					*																			
B. desidiosus										*												*							
B. festinus				*								*										*							
B. gilvus			*					*			*									*									
B. imminutus			*			*			*			*			*				*	*	*		*						
B. iugis																*			*	*	*		*						
Bact. castigatum				*									*																
Bact. idoneum																	*												*
Bact. lucrosum																					*								
Bact. paludosum						*																					*		
Ps. arguta			*																									*	
Ps. minuscula							*																					*	
Ps. mira									*				*			*		*						*					
B. biazoteus	(29)	*												*				*											
B. galbus	(29)				*						*													*					
B. gelidus	(29)							*																					
B. subalbus	(29)																	*								*			
Bact. fimi	(42)	*																				*							
Bact. flavigenum	(28)													*															
Ps. subcreta	(42)																						*		*				

The writer's extensive studies of the cultural characteristics of cellulose-destroying organisms has shown that a rapid destruction of cellulose occurs only when the culture medium is thoroughly aerated and contains an abundant supply of available nitrogen. It is also essential that fairly high temperatures be maintained. The thorough cultivation given most citrus soils in southern California insures thorough aeration. The surface soil to which the organic matter is usually added is generally well supplied with available nitrogen. The soil temperature even during the winter months is seldom below that at which a rapid multiplication of the cellulose-dissolving organisms takes place. In view of the above stated conditions, it would seem that the very rapid destruction of cellulose in these soils is probably due more to the very favorable cultural and climatic conditions which make possible the rapid multiplication of the cellulose-dissolving organisms in these soils.

New Species of Cellulose-Dissolving Bacteria

It is obvious that an adequate knowledge of cellulose decomposition in soils must be based upon a clear understanding of the character of the cellulose-dissolving micro-flora of soils. This knowledge can be obtained only by an arrangement of the organisms studied in a logical system of classification such as will make possible a comparative study of the forms described. In the establishment of the points of differentiation upon which separation may be based, there are of course many possible methods of procedure varying according to the points of resemblance which are selected as important.

In working out the description of new species of cellulose-dissolving bacteria, an attempt has been made to bring out the individual characteristics as concisely as possible. Many of the data called for by the card of the Society of American Bacteriologists seem to have little significance in the separation of members of this group. Moreover, in the isolation and classification of this group of organisms it has been found necessary to prepare several new varieties or culture media which are of especial importance in the classification of the cellulose-dissolving organisms, but would probably be of little imporance in the classification of ordinary saprophytic bacteria in soils. So far as we are able to determine none of the cellulose-dissolving organisms isolated have been previously described as saprophytic forms. In view of the above stated conditions and the fact that the power to dissolve cellulose forms a definite basis for the group, we believe that the classification of the cellulose-dissolving organisms can be most satisfactorily accomplished by the employment of only those media which are of especial importance in differentiating the members of this particular group and by using only those characters which remain constant through several sets of cultures.

Bacillus albidus, n. sp.

SOURCE: Soil from Tustin, California.

I. MORPHOLOGY.

1. Vegetative cells: Average dimensions 1 x .004 μ.
2. Endospores: None observed.
3. Flagella: 1 to 3 in number; 3 to 5 μ in length.
4. Staining reactions: Gram negative. Stain readily with the aniline dyes.

II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.
   Beef Agar: Scant, white, spreading growth.
   Potato agar: Abundant, white to grayish white growth, spreading over the entire slope.
   Peptone starch agar: Moderate, white to gray white growth.
6. *Potato cylinders:* No growth in 30 days.
7. *Gelatin stab:* Scant growth at surface and perceptible growth along the track of the needle; in 5 days. No liquefaction in 30 days.
8. *Beef broth*, 5 days. Not clouded.
9. *Litmus milk:* Reddened in 7 days, neither coagulated nor digested in 30 days.
10. Plate cultures.
    Ammonia cellulose agar, 15 days.
    Form: Colonies at the immediate surface of the medium are round, those located a little beneath the surface are irregularly round.
    Size: 8 to 12 mm.
    Enzymic zone: Clearing all within colony after 15 days. After 30 days the colonies show an enzymic zone of 1 to 2 mm.
    Elevation: Saucer shaped.
    Chromogenesis: Entire colony is vitreous with the exception of a thin, white rim.
    Internal structure: Indeterminate.
    Edge: Entire to undulate.
    Peptone starch agar, 5 days.
    Form: Colonies at the immediate surface are round, those slightly below the surface are irregularly round.
    Size: 2 to 3 mm.
    Enzymic zone: 1 to 1.5 mm.
    Elevation: Slightly convex.
    Chromogenesis: White to light grayish white.
    Internal structure: Coarsely granular; granules often arranged in clumps.
    Edge: Entire to undulate.
    Beef agar, 5 days.
    Form: Round.
    Size: 1 to 1.50 mm.
    Elevation: Convex.
    Consistency: Soft; colonies from 10 to 15 days old become brittle.
    Chromogenesis: By reflected light the colonies are light grayish white. By transmitted light they appear as semi-transparent glistening drops.
    Internal structure: Granular.
    Edge: Entire.

Potato agar, 5 days.
Form: Round.
Size: 2 to 3 mm.
Elevation: Convex.
Consistency: Soft, colonies from 15 to 20 days old become brittle.
Chromogenesis: Semi-transparent white, with pearl-like luster.
Internal structure: Granular.

11. *Filter paper broths,* 15 days. The paper is reduced to a thin, filmy grayish white mass which readily breaks up on slight agitation. The paper is readily attacked in solutions supplied with ammonium sulphate or peptone; but is much slower in solutions containing potassium nitrate or casein as the source of nitrogen.

III. Biochemical Features.

12. *Dunham's solution,* 10 days: No ammonia produced; no nitrite produced.
13. *Starch nitrate broth,* 10 days: No ammonia produced; no nitrite produced.
14. *Peptone nitrite solution,* 10 days: No indol produced.
15. *Carbohydrate broths,* 12 days: No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .50; Lactose, .20; Saccharose, .10; Malthose, .10; Glycerine, .10; Mannite, .10; Starch, .10.

Bacillus almus, n. sp.

Source: Soil from Arlington, California; Bonito, California, and Pasadena, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.2 x .5 μ.
2. Endospores: None observed.
3. Flagella: 1 to 5 in number; 3 to 4 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
Beef agar: Scant, white to grayish white growth. On slopes from 10 to 15 days old the growth becomes yellowish white.
Potato agar: Moderate, glistening, grayish white growth. After 10 days, the growth becomes yellowish.
Peptone starch agar: Moderate, glistening, grayish white growth, which becomes yellowish on old slopes.
6. *Potato cylinders:* No growth in 30 days.
7. *Gelatin stab:* Scant growth at surface and along track of the needle, in 5 days. No liquefaction in 30 days.
8. *Beef broth,* 5 days. Lightly clouded.
9. *Litmus milk:* Reddened in 6 days; neither coagulated nor digested in 30 days.
10. Plate cultures.
Ammonia cellulose agar, 15 days.
Form: Round.
Size: 4 to 6 mm. in 15 days; 6 to 8 mm. in 30 days.
Enzymic zone: 1 to 1.5 mm. in 15 days; in 25 days 3 to 4 mm.
Elevation: Saucer shaped.

Chromogenesis: Semi-transparent, grayish white after 15 days; older colonies become yellowish white with a narrow grayish white rim.

Internal structure: Colony is made up of fine loosely arranged granules. The rim of the older colonies is composed of large granules compactly arranged.

Edge: Entire.

Peptone cellulose agar, 15 days.

Form: Round or irregularly round.

Size: 5 to 7 mm. in 15 days; in 25 days colonies frequently attain a diameter of 20 mm.

Enzymic zone: 2 mm. in 15 days; 2.5 to 3.5 mm. in 30 days.

Elevation: Saucer shaped.

Chromogenesis: Central portion of colony 2 to 3 mm. in diameter is semi-transparent, grayish white; outer portion of colony is vitreous. The colony is usually surrounded by a narrow white rim.

Internal structure: Central portion of colony is granular; structure of the vitreous portion is indeterminate.

Edge: Entire to undulate.

Peptone starch agar, 5 days.

Form: Irregular. Those colonies at the immediate surface are round or nearly round, but those beneath the surface and the bottom colonies are quite irregular in outline.

Size: 2 to 3 mm.

Enzymic zone: 3 to 4 mm.

Elevation: Flat or very slightly convex.

Chromogenesis: The surface colonies show a small white nucleus, the remainder of the colony grayish white. The imbedded and bottom colonies are grayish to grayish white.

Internal structure: Granular.

Edge: Lacerate.

Beef agar, 5 days.

Form: Round.

Size: Surface colonies 1 to 1.5 mm.; bottom colonies 2 to 3 mm.

Elevation: Convex.

Consistency: Colony is soft during the first 10 days, after which it becomes brittle.

Chromogenesis: By reflected light the colonies are white to light grayish white. By transmitted light they are translucent light, smoky brown.

Structure: Granular.

Edge: Entire.

Potato agar, 5 days.

Form: Round.

Size: Surface colonies, 1 to 2 mm.; bottom colonies, 2 to 3 mm.

Elevation: Pulvinate.

Consistency: Butyrous after 5 days; somewhat viscous after 10 days.

Chromogenesis: Glistening, yellowish to grayish white.

Internal structure: Granular.

Edge: Entire.

11. *Filter paper broths,* 15 days. Paper reduced to a loose, felt-like mass which retains the pure white color of the paper. The structure of the paper has been entirely destroyed, as can be easily demonstrated by the slight agitation of the solution. The decomposition of the paper was less rapid with casein or potassium nitrate as the source of nitrogen than with peptone or ammonium sulphate.

III. Biochemical Features.

12. *Dunham's solution,* 10 days: No ammonia produced; no nitrite produced.
13. *Starch nitrate broth,* 10 days: No ammonia produced; no nitrite produced.
14. *Peptone nitrite solution,* 10 days: No indol produced.
15. *Carbohydrate broths,* 12 days: No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.30; Lactose, .80; Saccharose, 1.00; Maltose, 1.20; Glycerine, .40; Mannite, .00; Starch, .60.

Bacillus concitatus, n. sp.

Source: Soil from Barstow, California; Covina, California; Riverside, California.

I. Morphology.

1. Vegetative cells: Average dimensions, 1.2 x .5 μ.
2. Endospores: None observed.
3. Flagella: 1 to 3 in number; 3 to 4 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
   Beef agar: Abundant, flat, moist, yellowish white.
   Potato agar: Abundant, raised, moist, glistening, grayish white; old cultures become somewhat yellowish white.
   Peptone starch agar: Abundant, raised, frequently somewhat rugose, grayish white.
6. *Potato cylinders:* No growth in 30 days.
7. *Gelatin stab:* Moderate growth at surface and along stab in 5 days; slight napiform liquefaction after 30 days.
8. *Beef broth,* 5 days: Heavily clouded.
9. *Litmus milk:* Reddened in 4 days; no curdling or digestion apparent after 30 days.
10. Plate cultures.
    Ammonia cellulose agar, 15 days.
    Form: Surface colonies are round or irregularly round; bottom colonies spread out into irregular somewhat amoeboid growths.
    Size: Surface colonies are from 1 to 5 mm.; bottom colonies frequently attain a diameter of 15 mm.
    Enzymic zone: Surface colonies, 1 to 1.5 mm.; bottom colonies sometimes show no enzymic zone, but the colony is always more transparent than the surrounding medium, showing that some of the cellulose within the colony has been dissolved.
    Elevation: Flat or slightly depressed.
    Chromogenesis: Many of the colonies are almost pure white, while others show very thin brownish rings.
    Internal structure: Brownish rings coarsely granular; remainder of colony finely granular.
    Edge: Entire.

Peptone cellulose agar, 15 days.

Form: Surface colonies round; bottom colonies irregularly round.
Size: Surface colonies, 1 to 2 mm.; bottom colonies 12 to 15 mm.
Enzymic zone: Surface colonies, 2 to 2.5 mm.; bottom colonies, 1 mm. or less.
Elevation: Flat or very slightly convex.
Chromogenesis: Central portion of colony opaque white; outer portion, semi-transparent grayish white. Brownish rings sometimes apparent.
Internal structure: Central portion of colony coarsely granular, remainder of colony finely granular.
Edge: Usually entire, but some colonies throw out a thin film-like growth beyond the enzymic zone forming ear-like lobes.

Beef agar, 5 days.

Form: Round or irregularly round.
Size: Surface colonies 2 to 3 mm.; bottom colonies frequently spread over a large part of the plate.
Elevation: Decidedly convex.
Consistency: Soft; old colonies become slightly viscous.
Chromogenesis: White or light grayish white; bottom colonies frequently somewhat fluorescent.
Internal structure: Granular.
Edge: Entire to undulate.

Potato agar, 5 days.

Form: Round.
Size: Surface colonies 2 to 3 mm.; bottom colonies may attain a diameter of 10 mm.
Elevation: Distinctly convex; old colonies become somewhat umbilicate.
Consistency: Soft.
Chromogenesis: Glistening grayish white; some colonies show a white nucleus and rim.
Internal structure: Granular; nucleus is more coarsely granular than remainder of colony.
Edge: Entire.

11. *Filter paper broths,* 15 days. The paper is reduced to a disintegrated fibrous mass which retains its pure white color. The destruction takes place at about the same rate with ammonium sulphate, potassium nitrate or peptone as the source of nitrogen. With casein as a source of nitrogen the destruction of the paper is less rapid.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. No ammonia produced; no nitrite produced.
13. *Starch nitrate solution,* 10 days. No ammonia produced; nitrite produced.
14. *Peptone nitrite solution,* 10 days. Indol produced.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.80; Lactose, .85; Saccharose, 1.30; Maltose, 1.30; Glycerine, .45; Mannite, .00; Starch, 1.35.

Bacillus desiduosus, n. sp.

SOURCE: Soil from Covina, California, and Riverside, California.

I. MORPHOLOGY.

1. Vegetative cells: Average dimensions 1 x .4 μ.
2. Endospores: None observed.
3. Flagella: 1 to 3 in number; 3 to 5 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. CULTURAL CHARACTERISTICS.

5. Agar strokes, 5 days.
   Beef agar: Scant, flat, grayish white, filiform growth.
   Potato agar: Moderate, dry, cream-colored growth.
   Peptone starch agar: Abundant, grayish white growth.
6. *Potato cylinder:* No growth in 30 days.
7. *Gelatin stab:* Moderate grayish white growth at surface and along track of needle, in 5 days; no liquefaction in 30 days.
8. *Beef broth,* 5 days: Lightly clouded.
9. *Litmus milk:* Reddened in 3 days; neither coagulated nor digested in 30 days.
10. Plate cultures.
    Ammonia cellulose agar, 15 days.
    Form: Irregularly round.
    Size: Surface colonies are small, rarely becoming more than 1.5 mm. in diameter; bottom colonies frequently attain a diameter of 12 mm.
    Enzymic zone: 2 to 2.5 mm. in 15 days; 3 to 3.5 mm. in 25 days.
    Elevation: Slightly convex.
    Chromogenesis: Colony is gray white with the exception of a small white nucleus and a narrow white rim.
    Structure: Granular.
    Edge: Erose.
    Peptone cellulose agar, 15 days.
    Form: Irregularly round.
    Size: Surface colonies 1 to 2 mm.; bottom colonies may attain a diameter of 25 mm.
    Enzymic zone: Surface colonies 1 to 2 mm.; bottom colonies frequently show no enzymic zone until after 20 days.
    Elevation: Slightly convex.
    Chromogenesis: Surface colonies are semi-transparent, yellowish white. After 20 days' growth the surface and imbedded colonies become quite yellowish; bottom colonies remain grayish white.
    Internal structure: Granular.
    Edge: Lobate.
    Peptone starch agar, 5 days.
    Form: Surface and bottom colonies are round or irregularly round; imbedded colonies are flaky.
    Size: 1.5 to 2.5 mm.
    Enzymic zone: 1 to 1.5 mm. in 5 days; 2 to 2.5 mm. in 10 days.
    Elevation: Flat.
    Chromogenesis: Grayish white; some colonies show a small white nucleus.

Internal structure: Coarsely granular. The granules are frequently formed into large granular clumps.
Edge: Entire or undulate.

Beef Agar, 5 days.
Form: Surface colonies round; bottom colonies spread out into fern-like growths.
Size: Surface colonies 1 to 1.5 mm.; bottom colonies 12 to 15 mm.
Elevation: Slightly convex.
Consistency: Soft; old colonies are somewhat viscous.
Chromogenesis: By reflected light the colonies are grayish white; by transmitted light they appear as glistening semi-transparent drops.
Structure: Granular.
Edge: Entire.

Potato agar, 5 days.
Form: Round.
Size: 1 to 1.5 mm.
Elevation: Convex.
Consistency: Very soft; colony can be caused to spread over the medium by shaking the plate.
Chromogenesis: By reflected light the colonies are grayish white. By transmitted light they appear as glistening semi-transparent drops.
Structure: Granular.
Edge: Entire.

11. *Filter paper broths,* 15 days. Paper is reduced to a finely divided gray white mass which readily separates into minute fibrous particles on slight agitation. The paper is decomposed rapidly with ammonium sulphate, potassium nitrate, peptone or casein as the source of nitrogen.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. No ammonia formed; nitrite formed.
13. *Starch nitrate solution,* 10 days. No ammonia formed; nitrite formed.
14. *Peptone nitrite solution,* 10 days. Indol produced.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of gas (Fuller's Scale) with: Dextrose, .80; Lactose, .10; Saccharose, .00; Maltose, .60; Glycerine, .00; Mannite, .00; Starch, .20.

Bacillus festinus, n. sp.

Source: Soil from Banning, California; Fullerton, California; Whittier, California.

I. Morphology.

1. Vegetative cells: Average dimensions 2 x .6 μ.
2. Endospores: Form, elliptical; size, average dimensions .8 x .5 μ; germination, equatorial; rod, swollen.
3. Flagella: 1 to 3 in number; 4 to 6 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
Beef agar: Scant, flat, grayish white, spreading growth.

Potato agar: Abundant, grayish white, flat growth, usually spreading over the entire slope.

Peptone starch agar: Moderate, grayish white after 5 days, but in cultures from 6 to 10 days old the growth becomes a rich orange. The pigment diffuses through the medium very slowly.

6. *Potato cylinders:* No growth in 30 days.
7. *Gelatin stab:* Scant growth at surface and along track of the needle in 10 days. No liquefaction in 30 days.
8. *Beef broth:* Not clouded in 5 days.
9. *Litmus milk:* Reddened in 3 days; coagulated and digested in 25 days.
10. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Round.

Size: 10 to 12 mm. The colonies continue to grow after 15 days, and when kept in a moist chamber for 30 days the colonies frequently attain a diameter of 25 mm.

Enzymic zone: In young colonies the clearing is all within the colony; after 30 days the enzymic zone is frequently 2 to 3 mm.

Elevation: Saucer-shaped.

Chromogenesis: The central portion of the colony, usually 6 to 10 mm. in diameter, is semi-transparent grayish white. The remainder is vitreous with the exception of a thin white rim.

Internal structure: The central portion of the colony is made up of loosely arranged, coarse granules. The structure of the vitreous zone is indeterminate.

Edge: Entire.

Peptone cellulose agar, 15 days.

Form: Round.

Size: 5 to 6 mm. in 15 days; 10 to 12 mm. in 30 days.

Enzymic zone: 2 to 3 mm.

Elevation: Saucer-shaped.

Chromogenesis: Central portion of colony is transparent or semi-transparent grayish white. Outer portion of colony is semi-transparent yellowish white. Colony is usually surrounded by a thin yellowish white rim.

Internal structure: The colony is composed of fine granules loosely arranged.

Edge: Entire.

Peptone starch agar, 5 days.

Form: Surface colonies, round; imbedded and bottom colonies irregularly round.

Size: 15 to 25 mm.

Enzymic zone: 2 to 3 mm. in 5 days; 3.5 to 4 mm. in 10 days.

Elevation: Flat or very slightly convex.

Chromogenesis: Central portion of colony is a rich orange, outer portion grayish to yellowish white.

Internal structure: Consists of large granules frequently formed into clumps.

Edge: Entire to undulate.

Beef Agar, 5 days.

Form: Round.

Size: Surface colonies 1 mm. or less; bottom colonies 3 to 4 mm.

Elevation: Slightly convex.
Consistency: Butyrous, old colonies become brittle.
Chromogenesis: White nucleus, remainder semi-transparent, glistening, grayish white.
Internal structure: Finely granular with exception of nucleus which is made up of granular clumps.

Potato agar, 5 days.
Form: Round.
Size: Surface colonies 2 to 3 mm.; bottom colonies 4 to 5 mm.
Elevation: Convex; old colonies frequently become somewhat umbilicate.
Consistency: Butyrous.
Chromogenesis: Grayish to yellowish white. Sometimes shows brownish rings.
Internal structure: Finely granular.
Edge: Entire.

11. *Filter paper broths,* 15 days. Paper is very completely disintegrated into a grayish white felt-like mass, which readily separates into minute fibrous particles on slight agitation. The paper undergoes rapid decomposition when the nutrient solution contains inorganic nitrogen in the form of ammonium sulphate or potassium nitrate, and also when organic nitrogen is added in the form of peptone or casein.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. No ammonia produced; no nitrite produced.
13. *Starch nitrate solution,* 10 days. No ammonia produced; nitrite produced.
14. *Peptone nitrite solution,* 10 days. Indol produced.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .50; Lactose, .40; Saccharose, .00; Maltose, .65; Glycerine, .05; Mannite, .00; Starch, .60.

Bacillus gilvus, n. sp.

Source: Soil from Azusa, California; Chula Vista, California; Davis, California; Porterville, California; and Riverside, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.5 x .5 μ.
2. Endospores: None observed.
3. Flagella: 1 to 4 in number; 4 to 6 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
Beef agar: Scant, yellowish white, filiform growth.
Potato agar: Abundant, canary yellow, growth spreading over a large part of the slope.
Peptone starch agar: Abundant, grayish white, glistening growth which becomes somewhat yellowish after 10 days.
6. *Potato cylinders:* Abundant canary yellow in 5 days.
7. *Gelatin stab:* Moderate yellowish white growth at surface and along track of needle in 10 days; no liquefaction in 30 days.

8. *Beef broth,* 5 days: Slightly clouded.
9. *Litmus milk:* Reddened in 6 days; neither coagulated nor digested in 30 days.
10. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Round to irregularly round.
Size: 2 to 3 mm.
Enzymic zone: Entire colony semi-transparent; enzymic zone not more than 1 mm.
Elevation: Flat or slightly depressed.
Chromogenesis: Semi-transparent, grayish white, usually showing a small white nucleus.
Internal structure: Granular.
Edge: Entire to undulate.

Peptone cellulose agar, 15 days.

Form: Round.
Size: 2 to 4 mm.
Enzymic zone: 1.5 to 2 mm. in 15 days; 3 to 4 mm. in 25 days.
Elevation: Slightly concave.
Chromogenesis: Grayish white, frequently showing a small white nucleus, usually forms a thin grayish white semi-transparent rim beyond the enzymic zone.
Internal structure: Granular.
Edge: Entire.

Peptone starch agar, 5 days.

Form: Round to irregularly round.
Size: 2 to 3 mm.
Enzymic zone: 1 to 1.5 mm.
Elevation: Slightly convex.
Consistency: Soft, becoming brittle after 10 days.
Chromogenesis: Grayish to yellowish white. After 10 days the colonies become quite yellowish.
Internal structure: Granular.
Edge: Entire to undulate.

Beef Agar, 5 days.

Form: Round.
Size: Surface colonies 1 to 2 mm.; bottom colonies 3 to 3.5 mm.
Elevation: Convex.
Consistency: Soft.
Chromogenesis: After 3 days the colonies are grayish to yellowish white; the yellow color increases with the age of the colony and after 10 days they are distinctly yellow.
Internal structure: Coarsely granular. The granules are frequently formed into clumps.
Edge: Entire.

Potato agar, 5 days.

Form: Round.
Size: 2 to 3 mm.
Elevation: Convex.
Consistency: Butyrous.
Chromogenesis: Canary yellow; some colonies show brownish rings.
Internal structure: Granular.
Edge: Entire.

11. *Filter paper broths,* 15 days. The paper is reduced to a thin white filmy mass which breaks up into minute particles on slight agitation. The decomposition of the paper proceeds rapidly with ammonium sulphate, potassium nitrate, peptone or casein as the source of nitrogen.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. Ammonia formed; nitrite formed.
13. *Starch nitrate solution,* 10 days. No ammonia formed; nitrite formed.
14. *Peptone nitrite solution,* 10 days. Indol formed.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.20; Lactose, .75; Saccharose, .80; Maltose, 1.00; Glycerine, .40; Mannite, .00; Starch, 1.00.

Bacillus imminutus, n. sp.

Source: Soil from Highland, California; Berkeley, California; Corona, California; Redlands, California; Whittier, California; Santa Paula, California; Pasadena, California; Azusa, California; Fullerton, California; Porterville, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.5 x .2 μ. The vegetative cells pass quickly into involution forms which frequently attain a length of from 6 to 8 μ without increasing in thickness. The involution forms are commonly curved cells, frequently more or less fusiform.
2. Endospores: Form, round; average size, .5 μ; germination, polar. Rod is swollen during germination, giving the cell a drumstick appearance. On cellulose agar the spores appear in from 4 to 6 days.
3. Flagella: 1 to 5 in number; 3 to 5 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
   Beef agar: No growth.
   Potato agar: No growth.
   Peptone starch agar: No growth.
6. *Potato cylinders:* No growth in 30 days.
7. *Gelatine stab:* No growth in 30 days.
8. *Beef broth,* 5 days: No growth.
9. *Litmus milk:* No growth in 30 days.
10. Plate cultures.
    Ammonia cellulose agar, 15 days.
    Form: Round.
    Size: The size of the colonies is quite variable; after 15 days the diameter is usually between 12 and 15 mm. The colony continues to increase in size as long as the medium remains moist, and where plates can be kept free from molds a single colony may eventually cover the entire plate. The round form of the colony is maintained as long as the growth is unobstructed.

Enzymic zone: The entire colony is transparent. The enzyme does not clear the cellulose beyond the development of the colony.

Elevation: Young colonies are saucer-shaped. As the colony spreads the depression is less apparent.

Chromogenesis: Vitreous. Some colonies show a very narrow white rim. Old colonies frequently become a light transparent yellow.

Internal structure: Granular.

Edge: Entire.

Peptone cellulose agar, 15 days.

Form: Round.

Size: 10 to 12 mm. in 15 days; colonies continue to grow as long as the medium is kept moist. When the plate contains only a very few colonies the diameter may be 50 mm. or more in 30 days.

Enzymic zone: The entire colony is transparent with the exception of a very narrow white rim. The enzyme does not spread beyond the development of the colony.

Elevation: The young colonies are distinctly concave. As the colony becomes older the depression is less apparent.

Chromogenesis: Vitreous. Some colonies show a very narrow white rim. Old colonies frequently become a light trasparent yellow.

Internal structure: Indeterminate with the exception of the narrow white rim which is granular.

Edge: Entire.

Peptone starch agar: No colonies produced in 10 days.

Beef agar: No colonies produced in 10 days.

Potato agar: No colonies produced in 10 days.

11. *Filter paper broths,* 15 days. The paper is reduced to a very thin yellowish filmy mass, which disintegrates on very slight agitation. The paper is destroyed at about the same rate with ammonium sulphate, potassium nitrate or peptone as the source of nitrogen. A slower destruction of the paper occurs when nitrogen is supplied in the form of casein.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. No ammonia produced; no nitrite produced.
13. *Starch nitrate solution,* 10 days. No ammonia produced; no nitrite produced.
14. *Peptone nitrite solution,* 10 days. No indol produced.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 0.00; Lactose, 0.00; Saccharose, 0.00; Maltose, 0.00; Glycerine, 0.00; Mannite, 0.00; Starch, 0.00.

Bacillus iugis, n. sp.

Source: Soil from Lordsburg, California; Redlands, California; and San Fernando, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.4 x .4 μ.
2. Endospores: None observed.

3. Flagella: 1 to 3 in number; 3 to 4 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
   Beef agar: Scant, grayish white, filiform growth.
   Potato agar: Abundant, glistening, grayish white, filiform growth.
   Peptone starch agar: Moderate, grayish white, filiform growth.
6. *Potato cylinders,* 30 days: Scant, glistening, colorless growth when very heavily inoculated. Light inoculation produces no growth.
7. *Gelatin stab:* Moderate growth at surface and along track of needle in 5 days; napiform liquefaction in 30 days.
8. *Beef broth,* 5 days: Heavily clouded.
9. *Litmus milk:* Reddened in 5 days; neither coagulated nor digested in 30 days.
10. Plate cultures.
    Ammonia cellulose agar, 15 days.
    Form: Round.
    Size: 1.5 to 2.5 mm. in 15 days; 3 to 3.5 mm. in 25 days.
    Enzymic zone: Clearing sometimes all within colony after 15 days. After 20 days all colonies show an enzymic zone of 1 mm. or more.
    Elevation: Flat.
    Chromogenesis: Semi-transparent, light grayish white; sometimes contoured by light whitish lines.
    Internal structure: Granular.
    Edge: Entire.
    Peptone cellulose agar, 15 days.
    Form: Irregularly round.
    Size: 5 to 8 mm. in 15 days; no increase in size after 15 days.
    Enzymic zone: 2 to 3 mm. The zone continues to increase in width up to 30 days, in which time it is frequently 5 mm.
    Elevation: Slightly convex.
    Chromogenesis: Central portion of colony is white; the outer portion gray-white; sometimes forms a white nucleus and rim.
    Internal structure: Central portion of colony coarsely granular, outer portion finely granular.
    Edge: Undulate to lobate.
    Peptone starch agar, 5 days.
    Form: Irregularly round.
    Size: 1.5 to 2 mm. in 15 days; 2.5 mm. in 25 days.
    Enzymic zone: 1 mm. or less.
    Elevation: Capitate. (The colonies on starch agar are raised in a characteristic jelly-like mass.)
    Consistency: Gelatinous.
    Chromogenesis: Grayish white.
    Internal structure: Fine granules loosely arranged.
    Edge: Lancelate.
    Beef agar, 5 days.
    Form: Surface colonies round; imbedded colonies, lenticular.
    Size: 1.5 to 2 mm.
    Elevation: Convex.

Consistency: Soft; old colonies become somewhat gelatinous.
Chromogenesis: Small white nucleus, remainder semi-transparent grayish white.
Internal structure: Granular.
Edge: Entire.

Potato agar, 5 days.
Form: Surface colonies, round; bottom colonies, irregularly round.
Size: Surface colonies 1 to 1.5 mm.; bottom colonies 2.5 to 4 mm.
Elevation: Convex.
Consistency: Soft.
Chromogenesis: Grayish white, with a pearl-like luster.
Internal structure: Granular.
Edge: Entire.

11. *Filter paper broths,* 15 days. The paper retains something of its original structure; but shows many ragged holes where the fibers have been dissolved away. Very slight agitation is sufficient to disintegrate the paper mass completely. The decomposition takes place at about the same rate with ammonium sulphate or peptone as the source of nitrogen. The decomposition was much slower when casein or potassium nitrate was used.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. Ammonia produced; nitrite produced.
13. *Starch nitrate solution,* 10 days. No ammonia produced; nitrite produced.
14. *Peptone nitrite solution,* 10 days. No indol produced.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .80; Lactose, 1.10; Saccharose, 1.60; Maltose, 1.55; Glycerine, .45; Mannite, .20; Starch, 1.50.

Bacterium castigatum, n. sp.

Source: Soil from Banning, California; Glendora, California; and Wineville, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.2 x .4 μ.
2. Endospores: None observed.
3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

4. Agar strokes, 5 days.
Beef agar: Abundant, moist, glistening, grayish white growth.
Potato agar: Abundant, glistening, grayish white; becomes yellowish white after 10 days.
Peptone starch agar: Abundant, raised, somewhat rugose.
5. *Potato cylinders,* 30 days: No growth.
6. *Gelatin stab:* Moderate growth at surface and along track of needle in 6 days; no liquefaction after 30 days.
7. *Beef broth,* 5 days: Lightly clouded.
8. *Litmus milk:* Reddened in 3 days; neither coagulated nor digested in 30 days.

9. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Irregularly round.
Size: 1 to 1.5 mm.
Enzymic zone: 1 to 1.5 mm.; in 30 days the enzymic zone may attain a diameter of 2.5 mm.
Elevation: Slightly convex.
Chromogenesis: Opaque white or light grayish white.
Internal structure: Granular.
Edge: Undulate.

Peptone cellulose agar, 15 days.

Form: Irregularly round.
Size: 1 to 1.5 mm.
Enzymic zone: .5 to 1 mm.; in 30 days the enzymic zone may reach a diameter of 2 mm.
Elevation: Slightly convex.
Chromogenesis: White nucleus and rim, remainder of colony grayish white.
Internal structure: Nucleus and rim made up of coarse compact granules, remainder of colony finely granular.
Edge: Undulate.

Peptone starch agar, 5 days.

Form: Irregularly round.
Size: 7 to 10 mm.
Enzymic zone: 1 to 1.5 mm. in 5 days; 2.5 to 3 mm. in 10 days.
Elevation: Flat or slightly convex.
Consistency: Firm.
Chromogenesis: Grayish white cottony-like colony in 5 days; in 10 days colonies become distinctly grayish.
Internal structure: Coarsely granular.
Edge: Lancelate.

Beef agar, 5 days.

Form: Round.
Size: Surface colonies 1 to 1.5 mm.; bottom colonies 2 to 3 mm.
Elevation: Slightly convex.
Consistency: After 5 days colonies are soft; after 10 days, brittle.
Chromogenesis: Very small white nucleus, remainder of colony grayish white. Surface colonies exhibit a pearl-like luster.
Internal structure: Granular.
Edge: Entire.

Potato agar, 5 days.

Form: Surface colonies round; bottom colonies irregularly round.
Size: Surface colonies 1 to 2 mm.; bottom colonies 2 to 3 mm.
Elevation: Convex.
Consistency: After 5 days colonies are soft; after 10 days, butyrous.
Chromogenesis: Light grayish white, semi-transparent colonies with a pearl-like luster.
Internal structure: Made up of fine granules, loosely arranged.
Edge: Entire.

10. *Filter paper broths,* 15 days. Paper very completely disintegrated and reduced to a pulp-like mass which settles to the bottom of the flask. The paper is vigorously attacked in solutions containing ammonium sulphate, potassium nitrate, or peptone as the source of nitrogen. Casein appeared to be less favorable for the rapid development of this organism.

III. Biochemical Features.

11. *Dunham's solution,* 10 days. No ammonia formed; nitrite formed.
12. *Starch nitrate broth,* 10 days. No ammonia formed; no nitrite formed.
13. *Peptone nitrite broth,* 10 days. No indol produced.
14. *Carbohydrate broths.* No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.50; Lactose, 1.10; Saccharose, 1.00; Maltose, 1.45; Glycerine, .55; Mannite, .00; Starch, 1.40.

Bacterium idoneum, n. sp.

Source: Soil from Mentone, California; and Whittier, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.5 to .5 μ.
2. Endospores: None observed.
3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

4. Agar strokes, 5 days.

Beef agar: Scant, yellowish white, glistening, filiform growth; in 10 days growth becomes distinctly yellowish.

Potato agar: Abundant, moist, glistening, faint yellowish to glistening white; becomes distinctly yellowish in 10 days.

Peptone starch agar: Moderate, flat, white, filiform growth; becomes faintly yellowish in 10 days.

5. *Potato cylinders:* Abundant, moist, glistening, grayish white growth in 15 days.
6. *Gelatin stab:* Moderate, yellowish growth at surface and along track of needle in 10 days. Slight napiform liquefaction after 30 days.
7. *Beef broth,* 5 days: Turbid.
8. *Litmus milk:* Reddened in 3 days; neither coagulated nor digested in 30 days.
9. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Irregularly round.
Size: 1 to 1.5 mm.
Enzymic zone: 1 mm. or less after 15 days; after 30 days the enzymic zone has attained a diameter of 2 to 3 mm.
Elevation: Flat.
Chromogenesis: Opaque white or light grayish white.
Internal structure: The colony is made up of rather coarse granules compactly arranged.
Edge: Lobate.

Peptone cellulose agar, 15 days.

Form: Irregularly round.
Size: 1 to 1.5 mm.; maximum development is reached in 15 days.
Enzymic zone: .5 to 1.0 mm. in 15 days; 1.5 to 2 mm. after 30 days.
Elevation: Flat.

Chromogenesis: Opaque white to light grayish white.
Internal structure: Coarse granules, compactly arranged.
Edge: Lobate.

Peptone starch agar, 5 days.
Form: Irregularly round.
Size: 1 to 2 mm.
Enzymic zone: 1 to 1.5 mm. in 5 days; 2 to 2.5 mm. in 10 days.
Elevation: Convex; frequently somewhat pulvinate.
Consistency: After 5 days the colonies are soft; older colonies become somewhat viscous.
Internal structure: Granular; granules frequently arranged in clumps.
Edge: Lobate.

Beef agar, 5 days.
Form: Round.
Size: Surface colonies 1 to 1.5 mm.; bottom colonies 2 to 3 mm.
Elevation: Convex.
Consistency: Soft; becomes brittle after 10 days.
Chromogenesis: Grayish white pearl-like luster. By transmitted light the colonies appear as semi-transparent glistening drops.
Internal structure: Granular.
Edge: Entire.

Potato agar, 5 days.
Form: Surface colonies, round; imbedded and bottom colonies, irregularly round.
Size: 2 to 3 mm.
Elevation: Pulvinate.
Consistency: After 5 days colonies are soft; after 10 days, butyrous or brittle.
Chromogenesis: Reflected light, yellowish to grayish white; transmitted light, semi-transparent glistening drops.
Internal structure: Coarsely granular.
Edge: Entire.

10. *Filter paper broths,* 15 days. Paper is reduced to a thin limp sheet which falls apart on slight agitation. Solutions containing ammonium sulphate, potassium nitrate and peptone as the source of nitrogen show a rapid decomposition of the paper. Solutions containing casein showed only a slight decomposition of the paper even after 30 days' incubation.

III. Biochemical Features.

11. *Dunham's solution,* 10 days. No ammonia formed; nitrite formed.
12. *Starch nitrate broth,* 10 days. No ammonia produced; nitrite produced.
13. *Peptone nitrite solution,* 10 days. No indol produced.
14. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.60; Lactose, 1.20; Maltose, 1.40; Mannite, .00; Saccharose, 1.30; Glycerine, .70; Starch, 1.40.

Bacterium lucrosum, n. sp.

Source: Soil from Redlands, California; and Upland, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.3. x .4 μ.
2. Endospores: None observed.

3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

4. Agar strokes, 5 days.

Beef agar: Moderate, flat, grayish white; old cultures become somewhat irridescent.

Potato agar: Moderate, dirty yellowish white in 5 days; becomes more yellowish with age.

Peptone starch agar: Abundant, moist, grayish white in 5 days; becomes faintly yellowish in 10 days.

5. *Potato cylinder,* 30 days: No growth.
6. *Gelatin stab:* No growth after 30 days.
7. *Beef broth,* 5 days: Heavily clouded.
8. *Litmus milk:* No change in milk in 30 days.
9. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Irregularly round.

Size: 15 to 20 mm.; in 30 days the colonies frequently reach a diameter of 25 to 30 mm.

Enzymic zone: When there are only a few colonies on the plate, permitting rapid spreading, the clearing is all within the colony until after 30 days, when an enzymic zone usually develops. On crowded plates the colonies always show an enzymic zone of 1 mm. or more.

Elevation: Slightly concave.

Chromogenesis: Central portion of colony, usually 6 to 19 mm. in diameter, is grayish white; outer portion of colony is vitreous. The vitreous zone is usually surrounded by a thin white rim.

Internal structure: Colony is made up of medium-sized, loosely arranged granules.

Edge: Undulate.

Peptone cellulose agar, 15 days.

Form: Round.

Size: 2 to 3 mm. in 15 days; 3 to 4 mm. in 25 days.

Enzymic zone: 1 to 1.5 mm. in 15 days; 2 to 3 mm. in 25 days.

Elevation: Flat.

Chromogenesis: Nucleus and rim are white, remainder of colony semi-transparent grayish white.

Internal structure: Granular.

Edge: Entire.

Peptone starch agar, 5 days.

Form: Irregularly round.

Size: 3 to 4 mm.

Enzymic zone: .5 to 1 mm. in 5 days; 2 to 3 mm. in 10 days.

Elevation: Convex.

Consistency: Soft; after 10 days colonies become brittle.

Chromogenesis: Central portion of colony semi-transparent, glistening white; outer portion vitreous.

Internal structure: Granular.

Edge: Undulate.

Beef agar, 5 days.

Form: Surface colonies, round; imbedded colonies, lenticular; bottom colonies, irregularly round.

Size: 1 to 1.5 mm.

Elevation: Slightly convex.

Consistency: Soft; in 10 days growth becomes somewhat gelatinous.

Chromogenesis: Very small white nucleus; remainder of colony semi-transparent grayish white.

Internal structure: Granular.

Edge: Entire.

Potato agar, 5 days.

Form: Surface colonies, round; bottom and imbedded colonies, irregularly round.

Size: 1.5 to 2 mm.

Elevation: Convex.

Consistency: Butyrous after 5 days; somewhat gelatinous after 10 days.

Chromogenesis: Yellowish to grayish white; after 10 days colonies become quite yellowish.

Internal structure: Coarsely granular. Some colonies are grumose.

Edge: Entire.

10. *Filter paper broths,* 15 days. Paper is reduced to pulpy grayish white mass consisting of very short fibers which separate on slight agitation. The paper is decomposed rapidly in the ammonium sulphate and peptone broths, but more slowly in the broths containing casein or potassium nitrate.

III. Biochemical Characteristics.

11. *Dunham's solution,* 10 days. No ammonia produced; nitrite produced.
12. *Starch nitrate solution,* 10 days. No ammonia produced; no nitrite produced.
13. *Peptone nitrite solution,* 10 days. No indol produced.
14. *Carbohydrate broths.* No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .20; Lactose, .10; Saccharose, .00; Maltose, .15; Glycerine, .00; Mannite, .05; Starch, .15.

Bacterium paludosum, n. sp.

Source: Soil from Berkeley, California; Whittier, California.

I. Morphology.

1. Vegetative cells: Average dimensions 1.5 x .4 μ.
2. Endospores: Form, elliptical; size, 1.2 x .6 μ; germination, equatorial; rod, swollen. Abundantly produced on potato agar cultures 3 or 4 days old.
3. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

4. Agar strokes, 5 days.

Beef agar: Moderate, flat, grayish white growth.

Potato agar: Abundant, moist, glistening, grayish white growth.

Peptone starch agar: Moderate, flat, grayish white growth.

5. *Potato cylinder:* Very scant, glistening, colorless growth sometimes occurs on cylinders held in a moist chamber for 30 days, but ordinarily no growth is secured.
6. *Gelatin stab:* Moderate growth at surface and along track of needle in 6 days. After 30 days napiform liquefaction is observed.
7. *Beef broth,* 5 days: Lightly clouded.
8. *Litmus milk:* Reddened in 5 days; neither coagulated nor digested in 30 days.
9. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Surface colonies, round or irregularly round; bottom colonies frequently develop into fern-like growths.
Size: Surface colonies, 2 to 3 mm.; bottom colonies frequently attain a diameter of 8 to 10 mm.
Enzymic zone: 1 to 3 mm. in 15 days; 3 to 3.5 mm. in 25 days.
Elevation: Slightly convex.
Chromogenesis: Surface colonies show a small white nucleus; remainder of colony, gray-white; bottom colonies are fluorescent.
Internal structure: Coarsely granular.
Edge: Entire to undulate.

Peptone cellulose agar, 15 days.

Form: Irregularly round.
Size: Surface colonies 1.5 to 2.5 mm.; bottom colonies 3 to 5 mm.
Enzymic zone: 1.5 to 2 mm. in 15 days; 2.5 to 3 mm. in 30 days.
Elevation: Convex.
Chromogenesis: White to grayish white. Colonies sometimes show a white nucleus and rim.
Internal structure: Coarsely granular.
Edge: Lacerate.

Peptone starch agar, 5 days.

Form: Irregular; imbedded colonies frequently throw out spine-like growths.
Size: 1.5 to 2 mm.
Enzymic zone: 1.5 to 2 mm. in 5 days; 2.5 to 3.5 mm. in 10 days.
Elevation: Flat.
Chromogenesis: White to light grayish white in 5 days; in 10 days the colonies become dark gray.
Internal structure: Densely granular.
Edge: Lacerate.

Beef agar, 5 days.

Form: Surface colonies round; bottom colonies spread out into irregular growths.
Size: Surface colonies 1.5 to 2 mm.; bottom colonies 10 to 12 mm.
Consistency: Very soft in 5 days; brittle in 10 days.
Chromogenesis: Semi-transparent, glistening, gray-white; frequently form a small white nucleus, and many colonies are more or less concentric in structure. At an angle of 45° the colonies are fluorescent.
Internal structure: Granular.
Edge: Entire to undulate.

Potato agar, 5 days.

Form: Round.

Size: 2 to 3 mm.; bottom colonies are no larger than the surface colonies.

Elevation: Decidedly convex; in 10 days colonies frequently become somewhat unbilicate.

Consistency: Butyrous.

Chromogenesis: Semi-transparent, glistening, light grayish white to almost vitreous. Many colonies exhibit a pearl-like luster.

Internal structure: Finely granular.

Edge: Entire.

10. *Filter paper broths,* 15 days. Paper is reduced to a white pulp-like mass made up of very short disintegrated fibers which become distributed through the solution on slight agitation. The paper is decomposed very rapidly with ammonium sulphate, potassium nitrate, and peptone as the source of nitrogen. The decomposition takes place more slowly when casein is added as the source of nitrogen.

III. Biochemical Features.

11. *Dunham's solution,* 10 days. No ammonia produced; nitrite produced.
12. *Starch nitrate solution,* 10 days. No ammonia produced; no nitrite produced.
13. *Peptone nitrite solution,* 10 days. Indol produced.
14. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.10; Lactose, .80; Saccharose, 1.00; Maltose, 1.20; Glycerine, .40; Mannite, .05; Starch, 1.20.

Pseudomonas arguta, n. sp.

Source: Soil from Azusa, California; and Whittier, California.

I. Morphology.

1. Vegetative cells: Average dimensions .8 x .3 μ.
2. Endospores: None observed.
3. Flagella: 1 to 2 in number; 6 to 8 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.

Beef agar: Scant, grayish white, filiform growth.

Potato agar: Moderate, yellowish, glistening white.

Peptone starch agar: Scant, white to grayish white.

6. *Potato cylinders,* 30 days. No growth.
7. *Gelatin stab:* Moderate yellowish growth at surface and along the track of needle in 10 days; no liquefaction in 30 days.
8. *Beef broth,* 5 days: Clouded.
9. *Litmus milk:* Reddened in 4 days; neither coagulated nor digested in 30 days.
10. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Round.

Size: Surface colonies, 1 to 2 mm.; bottom colonies, 3 to 4 mm.

Enzymic zone: 1 mm. or less in 15 days; in 30 days the zone frequently becomes 2 or 3 mm.

Elevation: Slightly convex.
Chromogenesis: White nucleus and rim; remainder semi-transparent grayish white.
Structure: Grumose.
Edge: Entire.

Peptone cellulose agar, 15 days.
Form: Round.
Size: 8 to 12 mm. in 15 days; in 30 days the colonies frequently attain a diameter of 20 mm.
Enzymic zone: 1 to 2 mm.
Elevation: Slightly convex.
Chromogenesis: Central portion, usually from 5 to 7 mm. in diameter, is yellowish white. The central portion of the colony is surrounded by a vitreous zone, which in turn is surrounded by a light grayish white rim.
Internal structure: Granular.
Edge: Erose.

Peptone starch agar, 5 days.
Form: Irregularly round.
Size: 5 to 8 mm.
Enzymic zone: 1 to 2 mm. in 5 days; 3 to 4 mm. in 10 days.
Elevation: Flat.
Consistency: Soft in 5 days; older colonies become firm.
Chromogenesis: Central portion, usually 2 to 3 mm. in diameter, opaque white. The opaque portion of the colony is surrounded by a vitreous zone, which in turn is surrounded by a thin semi-transparent grayish white rim.
Internal structure: Granular.
Edge: Undulate.

Beef agar, 5 days.
Form: Round.
Size: Surface colonies, 1 to 1.5 mm.; bottom colonies, 2 to 3 mm.
Elevation: Slightly convex.
Consistency: Soft to butyrous.
Chromogenesis: Reflected light, grayish white; transmitted light, the colonies appear as semi-transparent glistening drops.
Internal structure: Granular.
Edge: Entire.

Potato agar, 5 days.
Form: Round.
Size: 1 to 2 mm.
Elevation: Convex.
Consistency: Very soft.
Chromogenesis: Grayish white, frequently develops brownish rings.
Internal structure: Granular.
Edge: Entire.

11. *Filter paper broths,* 15 days. Paper is reduced to a loose flocculent mass which disintegrates very readily on slight agitation. Paper is decomposed rapidly when the broths contain ammonium sulphate, potassium nitrate, peptone, or casein as the source of nitrogen.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. No ammonia formed; nitrite formed.
13. *Starch nitrate solution,* 10 days. No ammonia formed; no nitrite formed.
14. *Peptone nitrite solution,* 10 days. No indol formed.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, .30; Lactose, .10; Saccharose, .00; Maltose, .20; Glycerine, .00; Mannite, .00; Starch, .30.

Pseudomonas minuscula, n. sp.

Source: Soil from Bonita, California; Lordsburg, California; and Sanger, California.

I. Morphology.

1. Vegetative cells: Average dimensions, .9 x .5 μ.
2. Endospores: None observed.
3. Flagella: 1, rarely 2 in number; 3 to 4 μ in length.
4. Staining reactions: Gram positive. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
   Beef agar: Moderate, flat, grayish white, filiform growth.
   Potato agar: Abundant, moist, glistening, grayish to yellowish white.
   Peptone starch agar: Moderate, grayish white, filiform growth.
6. *Potato cylinders:* No apparent growth after 30 days, but potato is bleached along the track of the inoculating needle.
7. *Gelatin stab:* Moderate growth at surface and along track of needle in 6 days; slight napiform liquefaction after 30 days.
8. *Beef broth,* 5 days: Turbid.
9. *Litmus milk:* Reddened in 6 days; neither coagulated nor digested in 30 days.
10. Plate cultures.
    Ammonia cellulose agar, 15 days.
    Form: Round or irregularly round.
    Size: Surface colonies 1 to 2 mm.; bottom colonies may attain a diameter of 6 to 10 mm.
    Enzymic zone: 1.5 to 2 mm.
    Elevation: Slightly depressed.
    Consistency: Soft.
    Chromogenesis: Nucleus and rim are white, remainder of colony grayish white.
    Internal structure: Granular.
    Edge: Undulate.
    Peptone cellulose agar, 15 days.
    Form: Round or irregularly round.
    Size: 1 to 2 mm.; bottom colonies frequently attain a diameter of 10 mm.
    Enzymic zone: 1 to 1.50 mm.
    Elevation: Slightly convex.
    Consistency: Soft.
    Chromogenesis: White to grayish white.
    Internal structure: Granular.
    Edge: Erose.

Peptone starch agar, 5 days.
Form: Irregular, round.
Size: 1 to 1.5 mm.
Enzymic zone: 2 to 3 mm.
Elevation: Slightly convex.
Consistency: Firm.
Chromogenesis: White to light grayish white.
Internal structure: Granular.
Edge: Lacerate.

Beef agar, 5 days.
Form: Round.
Size: 1 mm. or less.
Elevation: Slightly convex.
Consistency: Butyrous; old colonies become brittle.
Chromogenesis: By reflected light colonies are gray white; by transmitted light they appear as light, semi-transparent smoky drops.
Internal structure: Finely granular.
Edge: Entire.

Potato agar, 5 days.
Form: Round.
Size: 1 to 2 mm.
Elevation: Convex.
Consistency: Soft.
Chromogenesis: Gray-white, sometimes showing concentric structure.
Internal structure: Granular.
Edge: Entire.

11. *Filter paper broths,* 15 days. Paper reduced to a felt-like grayish white mass which breaks up into small particles on very slight agitation. Paper destroyed more rapidly in solutions containing ammonium sulphate, peptone, or potassium nitrate than in solutions containing casein.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. No ammonia produced; nitrite produced.
13. *Starch nitrate broth,* 10 days, No ammonia produced; nitrite produced.
14. *Peptone nitrite solution,* 10 days. Indol produced.
15. *Carbohydrate broths.* No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.20; Lactose, 1.10; Saccharose, 1.00; Maltose, 1.10; Glycerine, .00; Mannite, .00; Starch, .90.

Pseudomonas mira, n. sp.

Source: Soil from Corona, California; Glendora, California; Monrovia, California.

I. Morphology.

1. Vegetative cells: Average dimensions, 1.6 x .4 μ.
2. Endospores: None observed.
3. Flagella: 1 in number; 4 to 6 μ in length.
4. Staining reactions: Gram negative. Stains readily with the aniline dyes.

II. Cultural Characteristics.

5. Agar strokes, 5 days.
Beef agar: Moderate, flat, grayish white, somewhat iridescent.

Potato agar: Abundant, grayish white; becomes grayish brown in 10 days.

Peptone starch agar: Abundant, moist, grayish white; in 10 days the growth at the bottom of the slope becomes flesh colored.

6. *Potato cylinder:* Moderate, grayish white, leathery growth in 15 days.
7. *Gelatin stab:* Good growth at surface and along track of needle in 6 days; no liquefaction in 30 days.
8. *Beef broth,* 5 days: Heavily clouded.
9. *Litmus milk:* Blued in 10 days; neither coagulated nor digested in 30 days.
10. Plate cultures.

Ammonia cellulose agar, 15 days.

Form: Round or irregularly round.
Size: 1 to 1.5 mm.
Enzymic zone: 1 to 2 mm. in 15 days; 3 to 4 mm. in 30 days.
Elevation: Slightly convex.
Chromogenesis: Surface colonies opaque white; bottom colonies semi-transparent grayish white.
Internal structure: Granular.
Edge: Erose.

Peptone cellulose agar, 15 days.

Form: Round to irregularly round.
Size: Surface colonies, 1 to 2 mm.; bottom colonies 6 to 8 mm.
Enzymic zone: 1 mm. or less in 15 days; 2 to 3 mm. in 30 days.
Elevation: Slightly convex.
Chromogenesis: Surface colonies have a small white nucleus, remainder of colonies grayish white.
Internal structure: Granular.
Edge: Entire to undulate.

Peptone starch agar, 5 days.

Form: Irregularly round.
Size: 2 to 3 mm.
Enzymic zone: 1.5 to 2 mm.
Elevation: Slightly convex.
Consistency: Firm.
Chromogenesis: White to light grayish white; sometimes shows a small white nucleus.
Internal structure: Granular.
Edge: Lacerate.

Beef agar, 5 days.

Form: Surface colonies are round; bottom colonies spread profusely.
Size: 2 to 3 mm.
Elevation: Convex.
Consistency: Soft to butyrous.
Chromogenesis: Small white nucleus, remainder gray-white.
Internal structure: Granular.
Edge: Lacerate.

Potato agar, 5 days.

Form: Round.
Size: 2 to 5 mm.
Elevation: Convex.

Consistency: Very soft.
Chromogenesis: Glistening, grayish white; surface colonies have a pearl-like luster.
Internal structure: Granular.
Edge: Lacerate.

11. *Filter paper broths,* 15 days. Paper attacked along the edge nearest surface of solution; in 20 days the paper is very completely disintegrated. The paper decomposed at about the same rate in solutions containing ammonium sulphate, potassium nitrate, peptone, or casein as the source of nitrogen.

III. Biochemical Features.

12. *Dunham's solution,* 10 days. Ammonia produced; no nitrite produced.
13. *Starch nitrate solution.* Ammonia produced; nitrite produced.
14. *Peptone nitrite broth,* 10 days. No indol produced.
15. *Carbohydrate broths,* 12 days. No gas produced. Per cent of acidity (Fuller's Scale) with: Dextrose, 1.25; Lactose, .50; Saccharose, 1.10; Maltose, 1.20; Glycerine, .30; Mannite, .25; Starch, 1.50.

Summary of Specific Characteristics of Cellulose-Dissolving Bacteria

The detailed description of the cellulose-dissolving bacteria known today are scattered through several publications. In the identification of newly isolated forms or in comparing the specific characteristics of described forms, it is obviously desirable that the more important morphological and cultural features of the cellulose-dissolving organisms known at this time be brought together in such a way as to afford a ready comparison. The more important morphological and cultural features of the cellulose-dissolving bacteria are briefly summarized in Table II. The biochemical reactions of the different species are summarized in Table III.

Provisional Key for Identifying and Comparing Species of Bacteria which Dissolve Cellulose

In order to facilitate further the identification and classification of cellulose-dissolving bacteria a diagrammatic key has been prepared. In the preparation of a key of this character, it is desirable to use single diagnostic features by means of which the organisms may be separated into smaller and smaller groups until all species are finally separated from each other. In such an arrangement, it is obvious that the features used must have a high degree of constancy. In the preparation of the following key, only those features which have remained constant through a number of cultures have been used and it is believed that when the key is used in conjunction with the data presented in Tables II and III, it will be found of much help in separating a particular organism from its congeners or in assigning it a provisional place in a system of classification.

Importance of Cellulose Destruction in Soils.

All organisms make up for the waste incurred in their vital activities by the consumption of chemical energy. This necessary energy is for the

TABLE II

COMPARATIVE SUMMARY OF THE MORE IMPORTANT MORPHOLOGICAL AND CULTURAL FEATURES OF CELLULOSE-DISSOLVING BACTERIA

		Morphology				Cultural features							
						Beef agar					Litmus milk		
		Dimensions in microns	Number of flagella	Spores	Involution forms	Luxuriant growth	Yellow chromogensis	Broth clouded	Gelatin liquefied	Growth on potato	Milk reddened	Milk blued	Coagulated or digested
B. albidus		1.0 x .4	1-3	—	—	—	—	—	—	—	+	—	—
B. almus		1.2 x .5	1-5	—	—	—	+	+	—	—	+	—	—
B. amylolyticus	(28)	3.5 x .7	10-16	+	—	+	—	+	+	—	+	—	—
B. aurogenus	(29)	1.4 x .4	1-3	—	—	+	+	+	+	+	+	—	+
B. bibulus	(42)	1.3 x .4	1-4	—	—	+	—	+	+	+	+	—	—
B. biazoteus	(29)	.8 x .5	1-3	—	—	+	+	+	+	+	+	—	—
B. caesius	(29)	1.5 x .4	1-2	—	—	—	—	+	+	—	+	—	+
B. cellaseus	(29)	1.2 x .5	1-3	—	—	—	—	—	+	—	+	—	—
B. concitatus		1.2 x .5	1-3	—	—	+	+	+	+	—	+	—	—
B. cytaseus		2.7 x .5	10-18	+	+	—	—	—	—	—	—	—	—
B. desidiosus		1.0 x .4	1-3	—	—	—	—	+	—	—	+	—	—
B. festinus		2.0 x .6	1-3	+	—	—	—	—	—	—	+	—	+
B. galbus	(29)	1.0 x .4	1-3	—	—	+	+	+	+	—	+	—	—
B. gelidus	(29)	1.2 x .4	1-3	—	—	+	—	+	—	+	+	—	+
B. gilvus		1.5 x .5	1-4	—	—	—	+	+	—	+	+	—	—
B. imminutus		1.5 x .2	1-5	+	+	—	—	—	—	—	—	—	—
B. iugis		1.4 x .4	1-3	—	—	—	—	+	+	+	+	—	—
B. pusilus	(29)	1.1 x .6	1-3	—	—	—	—	+	—	—	+	—	—
B. rossicus	(28)	1.2 x .3	1-5	—	+	+	—	+	+	+	—	+	—
B. subalbus		.8 x .4	1-3	—	—	+	—	+	—	—	+	—	—
Bact. acidulum	(29)	1.0 x .3	—	—	—	—	—	—	—	—	+	—	—
Bact. castigatum		1.2 x .4	—	—	—	+	—	+	—	—	+	—	—
Bact. fimi	(42)	.9 x .4	—	—	—	+	—	+	+	+	+	—	—
Bact. flavigenum	(28)	1.0 x .4	—	—	—	+	+	+	+	+	+	—	—
Bact. idoneum		1.5 x .5	—	—	—	—	+	+	+	+	+	—	—
Bact. liquatum	(42)	1.7 x .4	—	—	—	+	+	+	+	+	+	—	—
Bact. lucrosum		1.3 x .4	—	—	—	+	—	+	—	—	—	—	—
Bact. paludosum		1.5 x .4	—	+	—	+	—	+	+	+	+	—	—
Bact. udum	(29)	1.5 x .5	—	—	—	+	—	+	+	+	+	—	—
Ps. arguta		.8 x .3	1-2	—	—	—	—	+	—	—	+	—	—
Ps. effusa	(29)	1.7 x .4	1-6	—	—	+	+	+	+	+	—	+	+
Ps. minuscula		.9 x .5	1-2	—	—	+	—	+	+	—	+	—	—
Ps. mira		1.6 x .4	1	—	—	+	—	+	—	+	—	+	—
Ps. perlurida	(29)	1.0 x .4	1-3	—	—	+	—	+	+	+	+	—	+
Ps. subcreta	(42)	1.4 x .4	1-5	—	—	—	—	—	—	—	—	—	—
Ps. tralucida	(29)	1.2 x .6	1-2	—	—	—	—	+	—	—	+	—	—

most part derived from the oxidation of carbon. Green plants through the agency of their chlorophyll have the power of utilizing the radiant energy of the sunlight to decompose the carbon dioxide of the air and use it in their metabolic processes. These plants receive thereby not only the necessary energy for their own life, but store up an enormous quantity of potential energy upon which animals and those plants which do not contain chlorophyll are largely dependent. Moreover, the successful growth

TABLE III

COMPARATIVE SUMMARY OF THE BIOCHEMICAL FEATURES OF CELLULOSE-DISSOLVING BACTERIA

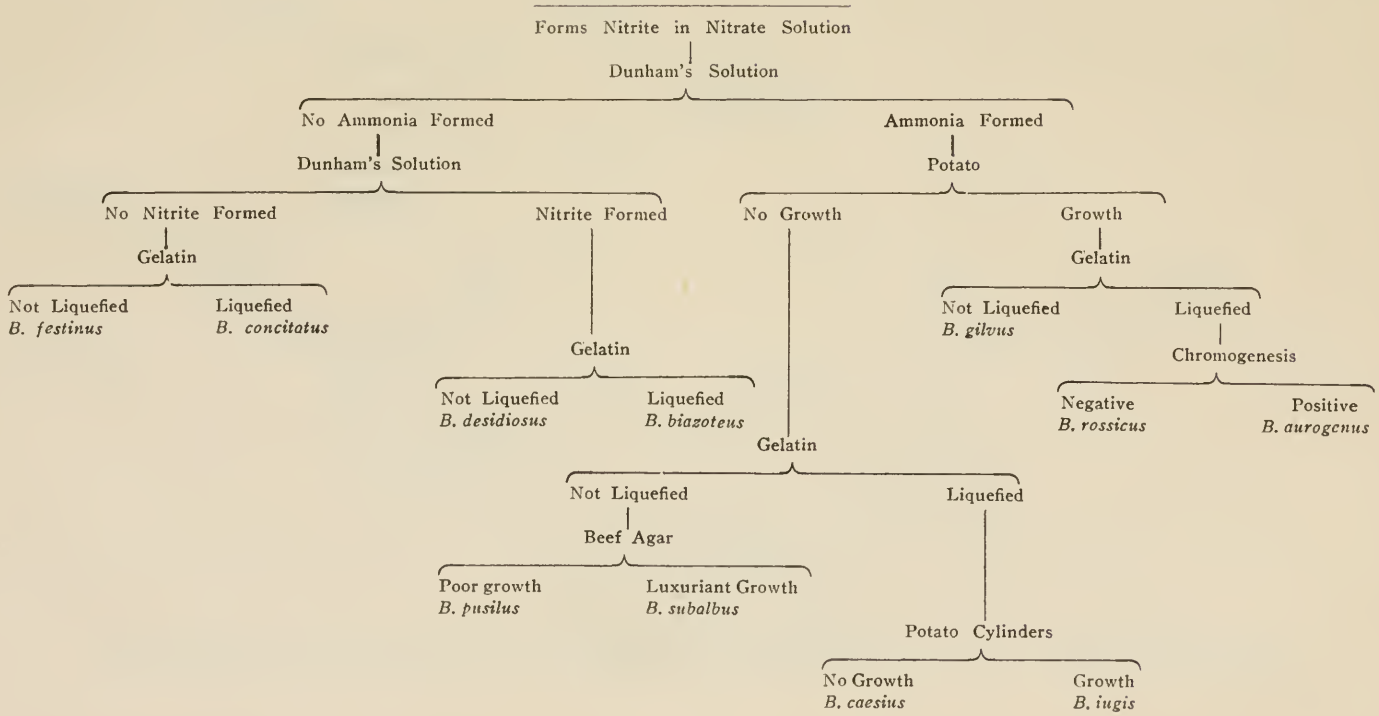
		Dunham's solution		Nitrate solution			Per cent acid produced in 12 days at 30° C.						
		Ammonia	Nitrite	Ammonia	Nitrite	Indol	Dextrose	Lactose	Saccharose	Maltose	Glycerine	Mannite	Starch
B. albidus		—	—	—	—	—	0.50	0.20	0.10	0.10	0.10	0.10	0.10
B. almus		—	—	—	—	—	1.30	0.80	1.00	1.20	0.40	0.00	0.60
B. amylolyticus	(28)	—	—	—	—	—	0.90	0.90	0.90	0.80	0.90	0.90	1.40
B. aurogenus	(29)	+	+	+	+	—	1.80	1.40	1.40	1.20	0.70	0.00	1.60
B. bibulous	(42)	+	+	—	—	+	1.80	1.30	1.50	1.50	0.40	1.20	2.00
B. biazoteus	(29)	—	+	—	+	—	2.00	1.10	1.00	0.90	0.50	0.00	1.50
B. caesius	(29)	+	+	+	+	—	1.90	1.50	1.40	1.10	0.50	0.20	1.40
B. cellaseus	(29)	—	+	—	—	—	1.40	0.40	1.40	0.80	0.30	1.10	1.20
B. concitatus		—	—	—	+	+	1.80	0.85	1.30	1.30	0.45	0.00	1.35
B. cytaseus		—	—	—	—	—	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. desidiosus		—	+	—	+	+	0.80	0.10	0.00	0.60	0.00	0.00	0.20
B. festinus		—	—	—	+	+	0.50	0.40	0.00	0.65	0.05	0.00	0.60
B. galbus	(29)	+	+	—	—	+	1.40	1.30	1.20	1.30	1.20	0.00	1.30
B. gelidus		+	+	—	—	—	1.20	1.20	0.80	1.20	0.40	0.00	1.40
B. gilvus		+	+	—	+	+	1.20	0.75	0.80	1.00	0.40	0.00	1.00
B. imminutus		—	—	—	—	—	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. iugis		+	+	—	+	—	0.80	1.10	1.60	1.55	0.45	0.20	1.50
B. pusilus	(29)	+	+	—	+	—	1.50	1.40	1.60	1.40	0.50	0.00	1.50
B. rossicus	(28)	+	—	—	+	—	−1.0	−1.4	−1.4	−1.6	−1.4	−1.5	−1.2
B. subalbus		+	+	—	+	—	1.60	1.00	1.40	1.20	0.70	0.20	1.40
Bact. acidulum	(29)	—	—	—	—	—	0.40	0.30	0.30	0.50	0.00	0.00	0.00
Bact. castigatum		—	+	—	—	—	1.50	1.10	1.00	1.45	0.55	0.00	1.40
Bact. fimi	(42)	+	+	—	+	+	1.60	0.90	1.60	1.40	0.80	0.00	1.60
Bact. flavigenum	(28)	—	+	—	+	—	1.00	0.90	0.70	0.90	0.30	0.10	1.40
Bact. idoneum		—	+	—	+	—	1.60	1.20	1.20	1.40	0.70	0.00	1.40
Bact. liquatum	(42)	+	—	—	+	+	1.30	1.00	1.30	1.20	0.20	0.00	1.40
Bact. lucrosum		—	+	—	—	—	0.20	0.10	0.00	0.15	0.00	0.05	0.15
Bact. paludosum		—	+	—	—	+	1.10	0.80	1.00	1.20	0.40	0.05	1.20
Bact. udum	(29)	—	+	+	+	—	1.40	1.30	1.40	1.20	0.00	0.00	1.40
Ps. arguta		—	+	—	—	—	0.30	0.10	0.00	0.20	0.00	0.00	0.30
Ps. effusa	(29)	+	—	—	+	—	2.10	−.50	−.70	0.60	0.30	0.20	1.20
Ps. minuscula		—	+	—	+	+	1.20	1.10	1.00	1.10	0.00	0.00	0.90
Ps. mira		+	—	—	+	—	1.25	0.50	1.10	1.20	0.30	0.25	1.50
Ps. Perlurida	(29)	+	—	—	—	—	1.80	1.50	1.50	1.20	0.60	1.50	2.00
Ps. subcreta	(42)	—	—	—	—	—	0.60	0.50	0.10	0.50	0.00	0.00	0.60
Ps. tralucida	(29)	—	+	—	+	—	1.30	0.70	1.60	1.00	0.40	0.20	1.60

of future generations of green plants is largely controlled by the liberation of this large store of potential energy through decomposition processes in the soil.

It is well known that through the agency of microörganisms, vegetable matter is gradually transformed into the complex mixtures ordinarily known as humus. In all cultivated soils, it is important to replenish from time to time the organic matter in the soil by the application of stable manure, green manure, etc. In semi-arid soils where the growth

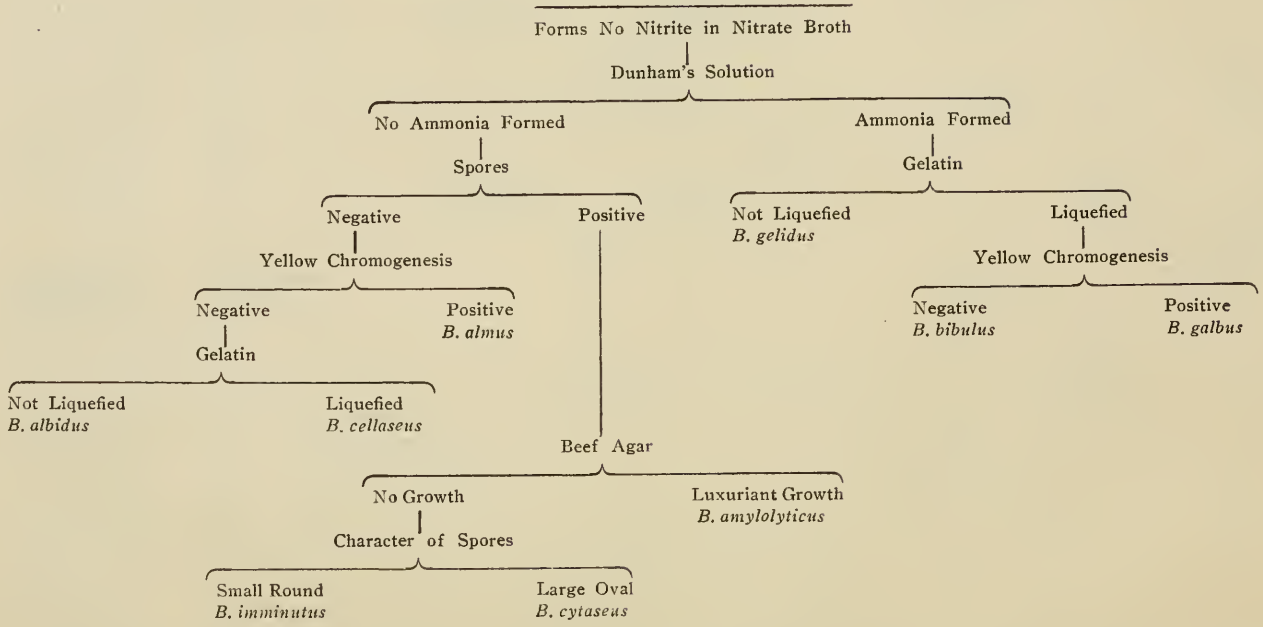
PROVISIONAL KEY FOR IDENTIFYING AND COMPARING SPECIES OF BACTERIA WHICH DISSOLVE CELLULOSE

GENUS *BACILLUS*, Part I

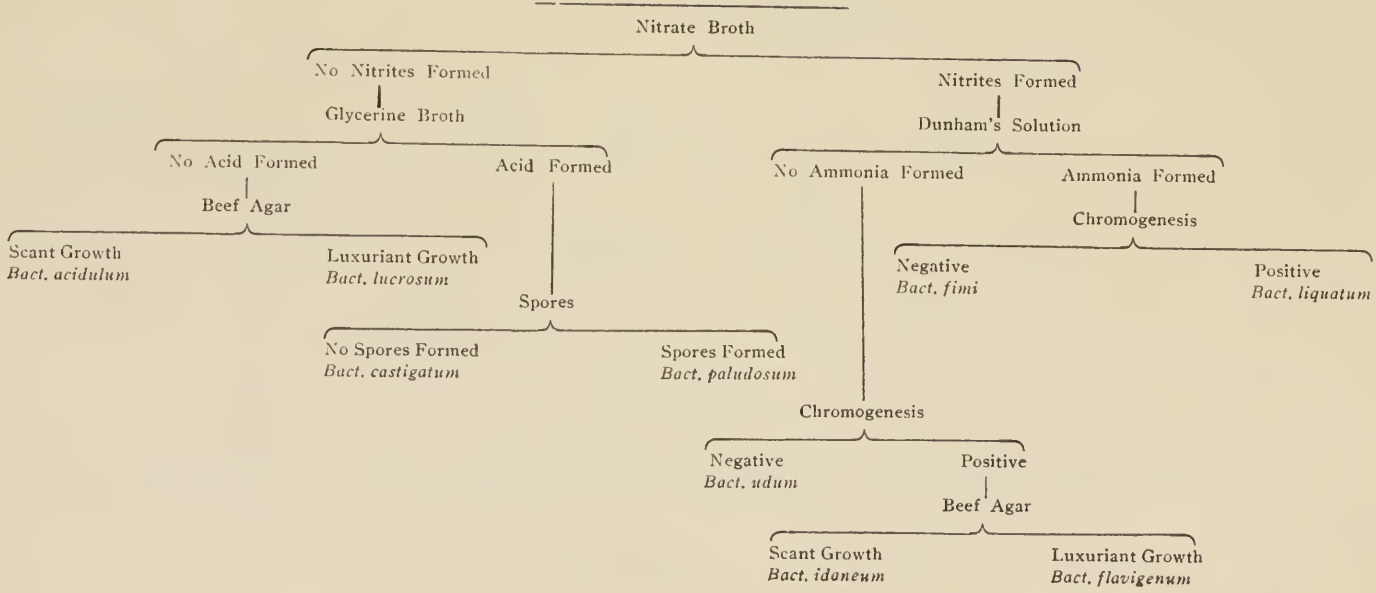


PROVISIONAL KEY FOR IDENTIFYING AND COMPARING SPECIES OF BACTERIA WHICH DISSOLVE CELLULOSE

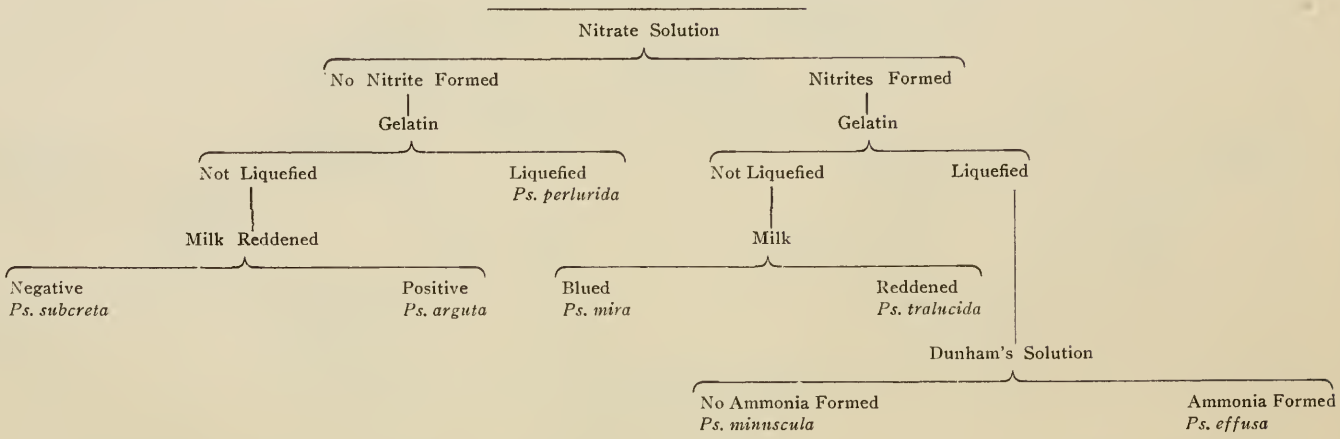
GENUS *BACILLUS*, Part II



PROVISIONAL KEY FOR IDENTIFYING AND COMPARING SPECIES OF BACTERIA WHICH DISSOLVE CELLULOSE
GENUS *BACTERIUM*



PROVISIONAL KEY FOR IDENTIFYING AND COMPARING SPECIES OF BACTERIA WHICH DISSOLVE CELLULOSE
GENUS *PSEUDOMONAS*



of native vegetation has been limited by the meager rainfall, the humus content of the virgin soil may be as low as 0.30 per cent or even less. When such soils are brought under intensive cultivation by means of irrigation, the scarcity of humus soon manifests itself by the development of injurious changes in the tilling qualities of the land. Many such lands soon fail to give satisfactory crops or respond to the application of commercial fertilizers unless the supply of organic matter is maintained by liberal applications of barnyard manure, green manures, etc.

As the larger part of carbonaceous matter added to soils in plant residues, stable manure, etc. is cellulose,—the gradual decomposition of the cellulose in soils in association with the nitrogenous compounds must play a very prominent rôle not only in maintaining the humus content of soils, but in securing the proper development of the many important biological processes. The humus content of the soil is considered by many to serve as the depository of the insoluble nitrogen of the soil which constitutes the reserve supply for crops. It is probable but not certain that this insoluble nitrogen through the process of nitrification furnishes the main nitrogen supply to plants. The fixation of atmospheric nitrogen in the soil is dependent upon the development of microörganisms which requires large quantities of organic carbon as food. During recent years, investigations by Koch (34), Pringsheim (63), and McBeth (42) have shown that cellulose may serve as a valuable source of energy for these organisms. However, cellulose is an extremely inert compound and the carbon contained therein can be utilized by the nitrogen fixing bacteria only after the cellulose has been converted into less refractory compounds by the cellulose-dissolving bacteria. It is obvious, therefore, that the work performed by these organisms is of fundamental importance in releasing the great store of energy locked up in cellulose. In view of the fact that the cellulose added to the soil represents a large amount of potential energy, the value of which depends upon the nature of the compounds formed in its decomposition, it becomes quite important to inquire into the nature of the compounds produced by the cellulose-dissolving bacteria. Earlier investigations by Popoff (61), Toppeiner (78), Hoppe-Seyler (25), Gayon (15), Deherain (13), Schloesing (74), Van Senus (76), Omeliansky (50), and others seemed to indicate that cellulose undergoes a direct gaseous fermentation in which a very large percentage of the carbon is converted into carbon dioxide and methane. Hoppe-Seyler was of the opinion that cellulose was dissolved according to the following formula:

(1) The hydration of the cellulose with the formation of a hexose,

$$C_6H_{10}O_5 + H_2O = C_6H_{12}O_6;$$ and

(2) The destruction of the carbohydrate with the formation of equal quantities of carbon dioxide and methane,

$$C_6H_{12}O_6 = 3CO_2 + 3CH_4.$$

If cellulose undergoes a direct gaseous fermentation in which a large part or all of the carbon is returned to the air in the first decomposition processes, the addition of cellulose to the soil would undoubtedly be of far less value than if the decomposition products formed by the cellulose-dissolving bacteria were non-volatile and remain in the soil, where they may assist in maintaining the humus content or may serve as a source of energy for important groups of bacteria, such as the nitrogen fixing organisms.

It is well known that fermentation processes in the soil resulting in a decomposition of the organic matter may give rise to large quantities of CO_2 and CH_4. However, we have been unable to show that these compounds are due to the activity of cellulose-dissolving bacteria. None of the cellulose-dissolving forms studied in our investigations give rise to gaseous products in cellulose or sugar solutions in which they make a luxuriant growth. Under natural conditions the compounds formed by the cellulose-dissolving bacteria will of course be seized upon by a host of other microörganisms and split up into simple compounds. In some soils the destruction may be extremely rapid and complete, resulting in the formation of little humus; under such conditions a very large percentage of the carbon in the cellulose is quickly liberated as CO_2. However, the CO_2 formed is presumably due in all cases to secondary fermentations by the action of the organisms upon the products produced by the cellulose-dissolving organism. Likewise, the organic acids noted by early investigators were, for the most part at least, presumably due to secondary fermentation and not to the action of the cellulose-dissolving forms.

The influence of the products of bacterial activity in rendering soluble various essential mineral constituents of the soil has come to be recognized as of considerable importance in maintaining the fertility of soils. It would seem that the insoluble compounds of potassium, phosphorus, magnesium, calcium, iron, sulphur, and even silicon may be rendered soluble through the production of carbon dioxide and organic acids which result from the decomposition of cellulose and other organic matter in soils. It is well known that limestones are quickly dissolved by carbonated waters, even granite and rocks related to it are attacked because of the feldspar minerals which contain potash, sodium and calcium together with aluminum. The results of this action would seem to be highly important in many western soils as the liberation of the aluminum results in the formation of clay which has an important influence on the physical condition of the soil, while the potassium is one of the essential nutrients of plant growth.

Phosphoric acid is so tenaciously held by most soils that ordinary leaching of the soil due to natural rainfall or irrigation would seem to bring very small amounts of this valuable substance into solution. The action of carbon dioxide upon the insoluble phosphorus compounds of the soil may proceed as follows:

$$Ca_3(PO_4)_2 + 2CO_2 + 2H_2O = Ca_2H_2(PO_4)_2 + Ca(HCO_3)_2$$

A large portion of the CO_2 resulting from the decomposition of cellulose or other carbonaceous materials in soils is ultimately returned to the atmosphere where it may be used over and over again in the manufacture of sugar, starches, cellulose, etc. in new generations of plants. If it were not for the activity of cellulose-dissolving organisms in the soil developing in association with gas producing organisms, the cycle of change to which carbon is subject would soon come to a standstill and the carbon supply of plants soon be depleted.

The importance of cellulose destruction in soil may then be summarized as follows:

1. The decomposition of cellulose under proper soil conditions and in association with the nitrogenous compounds of plant tissues makes possible the maintenance of the soil humus which is so essential in maintaining the proper tilling qualities of the land.

2. The cellulose added to the soil represents a large amount of potential energy which must have a marked stimulating effect on nitrogen fixation and many other important biological processes going on in the soil.

3. The decomposition of cellulose in soils, under proper conditions, results in the formation of large quantities of carbon dioxide. The action of carbonic acid in rendering available various mineral constituents of the soil is recognized as an important factor in the maintenance of soil fertility.

4. Through the decomposition processes, the carbon locked up in the cellulose is ultimately returned to the atmosphere, thus maintaining the carbon cycle and rendering the carbon supply for plants inexhaustible.

Summary

1. The cellulose agar plate method is the most satisfactory for isolating pure strains of bacteria, filamentous fungi or Actinomyces which have the power of dissolving cellulose.

2. In the preparation of precipitated cellulose for cellulose agar, the copper-ammonium-cellulose solution as well as the acid used should be very dilute. If either of the solutions are too concentrated, the precipitate is likely to be coarse, which not only makes it difficult to wash, but unsatisfactory for the preparation of culture media. A uniformly fine cellulose precipitate can be secured by diluting one part of the copper-ammonium-cellulose solution with forty parts of water and mixing with

a dilute hydrochloric acid solution, prepared by adding one part of concentrated acid to twenty parts of water.

3. Cellulose agar can be prepared from the cellulose in plant tissues by grinding the dry plant substances to a flour and isolating the cellulose in a pure state from the finely ground substance. Cellulose prepared in this way is quite as satisfactory for the preparation of cellulose agar as that prepared from filter paper in the ordinary way.

4. Twenty-five species of cellulose-dissolving bacteria have been grown on culture media containing cellulose prepared from alfalfa flour. All of the organisms plated to this medium dissolved the cellulose as readily as that prepared from filter paper.

5. All of the cellulose-dissolving organisms studied develop most rapidly in the presence of air, although more or less growth can be secured under anaerobic conditions.

6. Most of the cellulose-destroying bacteria grow well upon ordinary culture media. A few forms do not grow upon ordinary culture media, but only upon media containing cellulose.

7. The cellulose-dissolving bacteria assimilate nitrogen from organic as well as inorganic nitrogenous compounds. Many forms destroy cellulose rapidly when the culture medium contains nitrogen in the form of peptone, ammonium sulphate, potassium nitrate or casein. Peptone appears to be most favorable for the largest number of species, while casein is usually least favorable of the nitrogen compounds tested.

8. The quantity of acid formed in carbohydrate broths, in 12 days at 30° C. usually amounts to from 1 to 2 per cent on Fuller's scale, with dextrose, lactose, maltose, saccharose, and starch. The per cent of acidity in mannite and glycerine solutions is usually less than 1 per cent and in many instances no acid is formed from these substances.

9. Many species of cellulose-dissolving bacteria produce a small quantity of nitrite in Dunham's solution. The nitrite is presumably formed from the peptone. A starch nitrate broth free from peptone has therefore been used instead of the standard nitrate broth for determining the nitrate reducing power of these organisms.

10. Filamentous fungi play a much more important rôle in the destruction of cellulose in the humid soils of the eastern part of the United States than in the semi-arid soils of southern California.

11. Species of cellulose-dissolving Actinomyces have a wide distribution in soils and are unquestionably a factor in the destruction of cellulose in nature.

12. The very rapid destruction of cellulose which occurs in many soils of southern California is probably due to favorable climatic and cultural conditions which make possible the rapid development of the cellulose-dissolving organisms rather than to the unusually active nature of the cellulose-dissolving soil flora.

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